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Should treatment of low-level rifampicin mono-resistant tuberculosis be different?

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ABSTRACT

Background: Rifampicin resistant tuberculosis (RR-TB) was frequently detected in Suriname after the introduction of Xpert MTB/RIF in 2012. Subsequent phenotypic drug-susceptibility testing (DST) was not conclusive at that moment, while RR-TB patients treated with first-line tuberculostatics had good treatment outcome. In our study, we analysed this interesting observation.

Methods: We collected demographic and clinical characteristics and treatment outcome of TB patients from May 2012-December 2018 and performed a univariate and multivariate analysis to assess possible associations with resistance to rifampicin. Secondly, we conducted whole genome sequencing on all available *Mycobacterium tuberculosis* isolates that had a rifampicin resistance in the Xpert MTB/RIF test and performed phenotypic DST on selected isolates.

Findings: RR-TB was detected in 59 (9.6%) patients confirmed by Xpert. These patients were treated with rifampicin-containing regimens in most (88%) of the cases. In all 32 samples examined, a D435Y mutation in the *rpoB* gene was identified; only one isolate revealed an additional isoniazid mutation. Phenotypic DST indicated low-level rifampicin resistance. In multivariate analysis, the Creole ethnicity was a factor associated with rifampicin resistance (aOR 3.5; 95%CI 1.9–6.4). The treatment success rate for patients with RR-TB (78.0%) was comparable to the treatment outcome in non-RR-TB patients 77.8%.

Interpretation: This study confirms a low-level rifampicin mono-resistance in TB patients of Suriname. These patients could benefit from a first-line regimen with high dose rifampicin (or rifabutin), rather than from the lengthy treatment regimens for rifampicin-resistant and multi-drug resistant TB, a concept of stratified medicine also advocated for the treatment of TB.

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1. Introduction

Globally, an estimated 10 million people developed tuberculosis (TB) in 2018; half a million of them had rifampicin-resistant (RR) TB [1], of which 78% had also confirmed resistance to isoniazid (INH), and were hence classified as multidrug-resistant (MDR) TB.

The last decade brought large improvements in the diagnosis and treatment of RR/MDR-TB.[2] For instance, rifampicin resistance can now be diagnosed with rapid molecular tests and new effective second-line drugs as bedaquiline and linezolid, are now included in injection-

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free treatment regimens recommended by the World Health Organization (WHO).[3] The new diagnostic tests and drugs have become available to low and middle-income countries at special pricing schemes e.g. through support of the Global Fund to fight AIDS, TB and Malaria. Despite these advances, globally only 51% of patients with confirmed TB were tested for rifampicin resistance in 2018. Moreover, only 33% of all estimated RR/MDR-TB patients were enrolled in treatment, with a treatment success rate of 56%.[1]

Suriname is a country with a multi-ethnic population of 575,991 in 2018 (https://data.worldbank.org/country/Suriname) consisting of Hindustani (27%), Maroon (22%), Creole (16%), Javanese (14%), mixed (13%) or people with other ethnic background (8%) (https://statistics-suriname.org/nl/censusstatistieken-2012–2/). The TB notification rate was 30 per 100,000 population in 2018; WHO estimated the TB incidence at 38 per 100,000.[1] Before 2012, drug susceptibility testing (DST) was not routinely available in Suriname. Samples were sent abroad, and only two MDR-TB patients were diagnosed from 2005 till 2011. In May 2012, the Xpert® MTB/RIF test (Cepheid, Sunnyvale, CA, USA) was introduced in Suriname. The unexpected high proportion of rifampicin-resistant Xpert results has puzzled national and international TB experts for several years. Phenotypic drug susceptibility testing (DST) performed by one international laboratory in 2012 showed susceptibility to rifampicin, while re-analysis by the supranational reference laboratory several years later showed rifampicin resistance in these strains.[4] In 2018, 12.4% of confirmed cases in Suriname had RR-TB, which is the highest proportion in the WHO Region of the Americas.[1]

This study aims to identify characteristics of the RR-TB patients in Suriname, understand the resistance pattern found, search for similar strains in the Netherlands, a country with strong historical ties to Suriname, and identify possible implications for treatment recommendations.

2. Methods

2.1. Patients

In Suriname, TB patients are mandatory reported to the National Tuberculosis Program (NTP), which maintains an electronic national TB register. In our study, we included TB patients who were notified between May 2012 and December 2018. Patients were treated according to National Treatment Guidelines, which had been based on the 2010 Caribbean TB guidelines [5], with a standard regimen of isoniazid, rifampicin, ethambutol and pyrazinamide. Streptomycin and/or ciprofloxacin were added to the regimen in some patients who had previous TB treatment or RR-TB. Patients treated with a regimen without rifampicin were treated for 12–18 months. Patients, including those with RR-TB, were not actively followed up after treatment completion.

The following data were obtained from the register: sex, age, ethnicity, site of disease, smear results, Xpert results, drug-resistance, case type (new or previously treated), current TB treatment regimen, co-morbidities (HIV, diabetes mellitus), illicit drug use (marihuana or hard drugs) and treatment outcome. Treatment outcomes (including RR-TB) were based on the definitions and reporting framework for drug-sensitive TB [6], e.g. failure was defined if the sputum was microscopically or culture positive after 5 months treatment and cure if the smear was microscopically negative after 6 months treatment. A positive smear was graded as previously described.[7]

We compared demographic and clinical characteristics of Xpert-confirmed rifampicin-sensitive and rifampicin-resistant TB patients. Data were analysed with SPSS version 24 (SPSS, Inc., Chicago, IL, USA), including a univariate analysis to estimate the Odds Ratio (OR) and 95% Confidence Intervals (95%CI) and a multivariate regression analysis including those variables that attained a P value < 0.25 in the univariate analysis.

2.2. Mycobacterium tuberculosis isolates Suriname

In Suriname, samples of all TB patients are sent to the Central Laboratory, which accommodates the national TB reference laboratory, and performs culture (Löwenstein Jensen) and strain identification (SD BIOLINE TB Ag MPT64). Cultured *M. tuberculosis* isolates are stored at minus 80° Celsius.

In 2018, 32 stored Xpert rifampicin-resistant *Mycobacterium tuberculosis* isolates were re-cultured and sent to the National Tuberculosis Reference Laboratory of the National Institute for Public Health and the Environment (RIVM) in the Netherlands for further analysis. These isolates were from patients diagnosed in 2012 (n = 4), 2013 (n = 4), 2014 (n = 10), 2015 (n = 8), 2017 (n = 3) and 2018 (the first three RR-TB patients in that year). Rifampicin-resistant *M. tuberculosis* isolates from 2016 were not available for analysis. At the RIVM, whole genome sequencing (WGS) was applied to detect mutations associated with resistance and to determine the genetic distance between the isolates, as a proxy for epidemiological links between cases. Additionally, reverse line blot assays (GenoType MTBDR*plus*® Hain Lifesciences, Nehren, Germany) and phenotypic DST were performed on selected isolates.

2.3. Similar isolates in the Netherlands

We searched the Dutch TB laboratory database at the RIVM for isolates with the same mutation found in Suriname, and obtained data from the Dutch National TB Register of patients infected with *M. tuberculosis* isolates with this mutation. Isolates that were possibly identical with the strain found in Suriname were subjected to WGS.

2.4. Ethical consideration

Ethical approval for this study was obtained from the Human Scientific Research Ethic Committee of the Ministry of Health of Suriname. The Dutch National TB Registration Committee approved the use of the data from the Netherlands for this study.

3. Results

3.1. Patients

Between May 2012 and December 2018, a total of 978 TB patients were notified to the NTP in Suriname. The male–female ratio was 2.5 (696 male patients; 71%) with a median age of 42 years (interquartile range [IQR] 29–53). Patients' ethnicity was mostly Creole (32%), followed by Maroon (16%), Hindustani (15%) or mixed ethnicity (16%). Eighty-six percent (n = 838) of the patients had pulmonary TB; 599 had a smear positive sputum with microscopy (71% of pulmonary TB patients). Xpert was positive in 64% (n = 623) of the patients; whilst negative or not done in the remaining 36% (n = 355) of patients. Rifampicin resistance was determined in 59 (9.5%) of Xpert-positive patients (Table 1). Nine percent of patients (n = 91) were previously treated for TB. HIV status was known in 939 patients (96%) and HIV co-infection was present in 23% (n = 218) of tested patients. Diabetes mellitus was prevalent in 13% of the patients (n = 123) and illicit drug use was reported in 24% (n = 239) of the patients.

In univariate analysis, RR-TB patients were significantly more often in the age group 45–60 years (reference 15–24 years), had the Creole ethnicity, were previously treated for TB, had HIV co-infection and had documented illicit drug use, compared to patients with rifampicin-sensitive TB. Pulmonary RR-TB patients had more frequently microscopy-positive sputum smears (97%; 56/58) than pulmonary rifampicin-sensitive-TB patients (88%; 480/548) (p = 0.07). In multivariate analysis, patients with RR-TB were significantly more often of Creole ethnicity (aOR 3.5) (Table 2). HIV and illicit drug use were confounding factors related to Creole ethnicity: 32% (64/198) of all Xpert-positive Creole TB patients were HIV co-infected and 42% (84/

Table 1
Total number of tuberculosis (TB) and Xpert MTB/RIF rifampicin-resistant TB patients in Suriname, May 2012 - December 2018.

Year	Number of TB patients	Number of patients with confirmed diagnosis by Xpert MTB/RIF	Number of rifampicin-resistant TB (by Xpert MTB/RIF)	Proportion Xpert MTB/RIF-positive patients with rifampicin resistant TB
May-December 2012*	98	74	8	10.8
2013	141	91	8	8.8
2014	158	112	10	8.9
2015	150	78	8	10.3
2016	116	80	4	5.0
2017	136	83	8	9.6
2018	179	105	13	12.4
Total	978	623	59	9.5

Abbreviations: TB = tuberculosis
* The total number of tuberculosis patients in the year 2012 (January-December) was 135.

Table 2
Demographic and clinical factors of tuberculosis patients with positive Xpert MTB/RIF results in Suriname, May 2012 -December 2018.

	Patients with rifampicin sensitive TB (n = 564)		Patients with rifampicin-resistant TB (n = 59)		Unadjusted odds ratio		Adjusted odds ratio	
Characteristics	N	%	N	%	OR	CI	OR	CI
Male sex	420	74.5	48	81.4	1.5	0.8–3.0	0.9	0.4–2.0
Age group								
0–14 years	12	2.1	2	3.4	4.0	0.7–23.0	5.9	1.0–35.3
15–29 years	121	21.5	5	8.5	1		1	
30–44 years	167	29.6	16	27.1	2.3	0.8–6.5	1.4	0.5–4.2
45–59 years	187	33.2	32	54.2	4.1	1.6–10.9	2.4	0.9–6.8
60 years and above	77	13.7	4	6.8	1.3	0.3–4.8	1.0	0.3–4.1
Ethnicity*								
Creole	161	28.5	37	62.7	1		3.5	1.9–6.4
Other than Creole ethnicity	*(403)*	*(71.5)*	*(22)*	*(37.3)*	*(4.2)*	*(2.4–7.4)*	1	
Maroon	87	15.4	5	8.5	0.3	0.1–0.7		
Mixed	98	17.4	5	8.5	0.2	0.1–0.6		
Hindustani	85	15.1	7	11.9	0.4	0.2–0.8		
Javanese	64	11.3	4	6.8	0.3	0.1–0.8		
Amerindian	58	10.3	1	1.7	0.1	0.0–0.6		
Other/Unknown	11	2.0	0	0.0	0.0			
Pulmonary TB	548	97.2	58	98.3	1.7	0.2–13.0		
Previous TB treatment	46	8.2	12	20.3	2.9	1.4–5.8	2.0	1.0–4.3
HIV positive	95	16.8	20	33.9	2.5	1.4–4.5	0.8	0.4–1.5
Diabetes mellitus	98	17.4	7	11.9	0.6	0.3–1.5		
Illicit drug use	155	27.5	27	45.8	2.2	1.3–3.8	0.7	0.4–1.2

Abbreviations: CI = 95% confidence interval; HIV = human immunodeficiency virus; OR = odds ratio; TB = tuberculosis
* Creole: descendant of African slaves that may also have European and other ancestors; Maroon: descendant of escaped African slaves; Hindustani: descendant of contract laborers from what was then British India; Javanese: descendant of contract laborers predominantly from Java, Indonesia [33].

198) had documented illicit drug use versus respectively 12% and 23% of patients with other ethnicities.

Treatment outcome was successful (cured/completed) in 74.6% of all TB patients, in 77.8% of patients with Xpert rifampicin-sensitive TB and in 78.0% of patients with Xpert diagnosed RR-TB (Table 3). Second-line drugs (ciprofloxacin (n = 15), streptomycin (n = 2), or both ciprofloxacin and streptomycin (n = 20)) were added to the standard first-line treatment regimen in 37 patients; 21 of these patients had rifampicin-sensitive TB (4% of all rifampicin-sensitive TB patients) and 16 had RR-TB (27% of all RR-TB patients). Fifty-two (88%) RR-TB patients were treated with rifampicin-containing first-line regimens (11 out 52 were also treated ciprofloxacin and/or streptomycin), five (8%) patients were treated with the first-line drug regimen without rifampicin but to which was added ciprofloxacin and/or streptomycin, and two (3%) patients did not start treatment.

In total, 12 RR-TB patients were previously treated for TB (one person was treated for TB two times and another person three times) (Table 4). Three of these patients restarted treatment after discontinuing the treatment in the first episode (two had RR-TB in the first episode, the other one had a diagnosis before 2012) and nine completed treatment in the first episode (two diagnosed with RR-TB before, one with rifampicin-sensitive TB, five had a TB diagnosis before 2012 with an unknown resistance pattern, and in one patient DST was not done in the previous episode).

3.2. Whole genome sequencing (WGS) and additional laboratory tests

WGS revealed a D435Y mutation in the *rpoB* gene in all 32 rifampicin-resistant *M. tuberculosis* isolates. One isolate had an additional resistance mutation in the *inhA* gene S94A, but not in the more common *inhA* promoter gene region, and by definition labelled as MDR. None of the remaining 31 isolates had additional first-line drug resistance-associated mutations in the *katG, inhA, fabG1, ahpC, embA, embB, pncA* or *rpsA* genes. The 32 isolates were all linked together by WGS in one genetic cluster with $\leq$ 12 single nucleotide polymorphisms (SNPs) difference to each other.

The GenoType MTBDR*plus* analysis of 14 isolates (from 2015 to 2018) showed loss of hybridization of wild type probes 3 and 4, and no hybridization of the known and most frequently reacting mutation probes. Phenotypic DST, done by the Middlebrook 7H10 agar proportion method [8], indicated a MIC (minimal inhibitory concentration) for rifampicin of 1–5 mg/L, known as low level resistance (cut-off is 1 mg/L) and showed susceptibility to rifabutin for the three isolates tested (all patients diagnosed in 2018). These three isolates were also tested in the MGIT and were found susceptible to rifampicin.

3.3. D435Y isolates and patients in the Netherlands

The Dutch national TB laboratory database contained 15

Table 3
Treatment results of tuberculosis patients in Suriname, May 2012 - December 2018.

	All TB patients		TB patients with positive Xpert MTB/RIF with negative test for rifampicin resistance		TB patients with positive Xpert MTB/RIF with positive test for rifampicin resistance **	
	N	%	N	%	N	%
Successful (cured/completed)	730	74.6	439	77.8	46	78.0
Treatment failed	2	0.2	2	0.4	1	1.7
Died	136	13.9	59	10.5	4	6.8
Lost to follow-up	68	7.0	42	7.4	6	10.2
Treatment stopped (medical reason)	21	2.1	5	0.9	1	1.7
Not evaluated*	21	2.1	17	3.0	1	1.7
Total	978		564		59	

Abbreviations: TB = tuberculosis

* Not evaluated: patients transferred out and patients whose treatment outcome was unknown.

** 41 rifampicin-resistant tuberculosis (RR-TB) patients were treated with first-line drugs including standard dose rifampicin; 36 (87.8%) had a successful treatment outcome (median days treatment: 198; interquartile range [IQR] 187–222). 11 RR-TB patients were treated with first-line drugs including standard dose rifampicin, and in addition with ciprofloxacin (n = 4), streptomycin (n = 1) or ciprofloxacin and streptomycin (n = 6); 6 (55%) had a successful treatment outcome (median days treatment: 289; IQR 280–304). Five RR-TB patients were treated with first-line drugs without rifampicin, and in addition with ciprofloxacin (n = 2) or ciprofloxacin and streptomycin (n = 3); 4 (80%) had a successful treatment outcome (median days treatment: 381; IQR 362–447). Two RR-TB patients did not start treatment: one died before treatment and one was not evaluated.

Table 4
Patients with rifampicin-resistant tuberculosis in Suriname who were previous treated for tuberculosis, May 2012 - December 2018.

	Previous episode(s)			Last episode (RR-TB)	
Number	Year (s)	DST	Treatment result	Year	Treatment result
1	2009	Unknown	Lost to follow-up	2012	Cured
2	2009	Unknown	Cured	2012	Cured
3	2012	RR	Lost to follow-up	2013	Lost to follow-up
4	2009	Unknown	Cured	2013	Cured
5	2012	RR	Lost to follow-up	2013	Lost to follow-up
6	2000	Unknown	Cured	2014	Cured
7	2013	RR	Cured	2014	Cured
8	2013	RR	Cured	2015	Cured
9	2008 2015	Unknown ND	Lost to follow-up Cured	2017	Died
10	2013	Sensitive	Cured	2017	Lost to follow-up
11	2014 2015 2016	ND ND ND	Completed Lost to follow-up Cured	2017	Lost to follow-up
12	2010	Unknown	Cured	2018	Cured

DST = drug susceptibility test; ND = not determined; RR = rifampicin resistance; TB = tuberculosis

M. tuberculosis isolates with the same D435Y mutation found in Suriname (Table 5). The isolates were from patients born in Europe (n = 6), Suriname (n = 5), Africa (n = 3) and Asia (n = 1). The isolates from the 10 non-Suriname-born patients were resistant to isoniazid and 8 out of 10 susceptible to rifabutin; patients were treated accordingly (Table 5). The isolates from the 5 Suriname-born patients were all susceptible to isoniazid, rifampicin-susceptible in two cases (both tested by MGIT-DST) and low-level rifampicin-resistant in three cases (one patient with recurrent TB was counted twice; all these isolates were tested by Middlebrook 7H10). The two patients with rifampicin-sensitive TB completed standard first-line treatment, including standard dose rifampicin. One RR-TB patient was treated for 6 months with a first-line regimen, containing rifabutin instead of rifampicin, to which the strain was susceptible. The patient diagnosed twice with RR-TB first completed 12 months treatment with isoniazid, ethambutol, moxifloxacin and 2 months pyrazinamide, but developed RR-TB again after ten years. This second time, he was treated for 6 months with the first-line drug regimen, with rifabutin, to which the strain was susceptible, replacing rifampicin. Genotypically, all five *M. tuberculosis* isolates of the Suriname-born patients in the Netherlands clustered in the WGS with those of the 32 patients diagnosed in Suriname.

4. Discussion

Our study shows that rifampicin-resistant TB in Suriname is caused by a *M. tuberculosis* isolate with a D435Y mutation in the *rpoB* gene, known to be associated with a low-level resistance for rifampicin.[9–12] Only one patient had also an uncommon mutation coding for isoniazid resistance and hence had MDR-TB; all other RR-TB patients had rifampicin mono-resistant isolates. RR-TB patients were mainly treated with rifampicin-containing regimens. Treatment outcome was similar in patients with rifampicin-sensitive and RR-TB, with a successful treatment completion rate of 78% in both groups. Patients were not actively followed up after treatment completion and only identified with recurrent TB if they presented with symptoms and were diagnosed. Patients with isolates harbouring the D435Y mutation were also identified in the Netherlands, but only the patients born in Suriname had an infection with a rifampicin mono-resistant strain. These patients had been treated either as rifampicin-sensitive TB, or with first-line regimens including rifabutin.

Rifampicin resistance in *M. tuberculosis* is mostly caused by an undisputed mutation in the *rpoB* gene, most commonly the S450L mutation. The D435Y mutation described in our study is a mutation with disputed drug-resistance and characterised by a substitution of the amino acid asparagine (Asp/D) by tyrosine (Tyr/Y) in codon 516.[13] In the previous classification with *E. coli* codon numbering, this mutation was known as D516Y or GAC516TAC and renamed with the H37Rv numbering to D435Y. Studies investigating the accuracy of current laboratory tests to identify resistance in D435Y mutation isolates concluded that low level rifampicin resistance is often missed in the single point concentration MGIT-DST, while it is identified by MIC methods like Middlebrook 7H10 and Löwenstein-Jensen and by molecular test such as Xpert and MTBDR*plus*[9–11,14] Other studies concluded that the rifampicin resistance is often borderline or low level.[12,15–19] Almost all studies concluded that isolates with a D435Y mutation are susceptible to rifabutin.[10,15–17,20]

The Suriname NTP had difficulties in the interpretation of the rifampicin resistance detected by the Xpert test. In 2012, six Xpert rifampicin-resistant *M. tuberculosis* isolates were transported to an international laboratory, where MGIT-DST and 7H10 Middlebrook MIC method revealed susceptibility to rifampicin in all isolates. In 2016, the supranational reference laboratory of Massachusetts re-analysed these isolates using an agar proportion method and found rifampicin resistance in four of the six isolates. Furthermore, it performed PCR-mediated direct DNA sequencing of the *rpoB* gene on these six isolates and on 10 lysates of Xpert rifampicin-resistant *M. tuberculosis* isolates from 2014, revealing the D435Y mutation in 14 of the 16 samples.[4]

WHO's treatment advise on rifampicin mono-resistant TB has changed over time. In the first guidelines on drug-resistant TB, a 12–18 months regimen with isoniazid, ethambutol and a fluoroquinolone, and

Table 5
Patients with *Mycobacterium tuberculosis* isolates with a D435Y mutation in the Netherlands, 2003–2018.

Number	Year	Country/region of birth	Drug-susceptibility test results				Treatment regimen
			INH	RIF (MIC)	RIF(MGIT; 1 mg/l)	RBT	
1.	2003	Eastern-Europe	R	R (>5 mg/L)	ND	S	2nd line + RBT
2.	2003	Netherlands	R	R (2 mg/L)	ND	S	2nd line + RBT
3.	2003	European Union	R	R (2 mg/L)	ND	S	2nd line + RBT
4.	2005 [a]	Suriname	S	R (2 mg/L)	ND	ND	1st line (without RIF/RBT) + moxifloxacin
5.	2006	Netherlands	R	S (0.5 mg/L)	ND	S	1st line (including standard dose RIF) + moxifloxacin
6.	2010 [a]	Suriname	S	ND	S	ND	1st line (including standard dose RIF)
7.	2011	European Union	R	R (2 mg/L)	R	S	2nd line + RBT
8.	2011	Africa	R	R (2 mg/L)	ND	S	2nd line + RBT
9.	2012	Africa	R	R (2 mg/L)	ND	R	2nd line (without RIF/RBT)
10.	2012 [a]	Suriname	S	ND	S	ND	1st line (including standard dose RIF)
11.	2013	Asia	R	ND	R [b]	R [b]	2nd line (without RIF/RBT)
12.	2014 [a]	Suriname	S	R (2 mg/L)	ND	S	1st line (including RBT)
13.	2016	Eastern-Europe	R	ND	S	S	2nd line + RBT
14.	2016	Africa	R	S (0.5 mg/L)	S	S	2nd line + high dose RIF
15.	2017 [a]	Suriname	S	R (5 mg/L)	S	S	1st line (including RBT)

Abbreviations: INH = isoniazid; MGIT = Mycobacteria Growth Indicator Tube; MIC = minimal inhibitory concentration (by Middlebrook 7H10 agar proportion method); ND = not determined; R = resistant; RBT = rifabutin; RIF = rifampicin; S = susceptible.

[a] Patients 4, 6, 10, 12 and 15 had clustering *M. tuberculosis* isolates in Whole Genome Sequencing (≤12 single nucleotide polymorphism difference).

[b] The *M. tuberculosis* isolate had two mutations in the *rpoB* gene, i.e. D435Y and S450L.

pyrazinamide for at least two months, was recommended.[21] These guidelines also advised a retreatment regimen with first-line drugs and streptomycin ("Category 2 regimen") for patients with relapse or return after loss to follow-up. In 2011, WHO published an update stating that the detection of rifampicin resistance by Xpert test usually suffices to prescribe second-line TB regimen [22], but also cautioned for situations in which the Xpert test has a low predictive value and advised that results need to be confirmed by phenotypic DST or line probe assay. The update also noted that a potential harm from placing all rifampicin-resistant patients on an MDR-TB regimen, was the exclusion of isoniazid from their treatment, thus depriving them of a safe and useful bactericidal drug.[22] A systematic review in 2016 identified only three studies reporting treatment outcomes for patients with RR-TB, preventing a *meta*-analysis.[23] Since 2016, WHO guidelines on drug-resistant TB recommend similar treatment regimens for patients with RR-TB and MDR-TB.[3,24] The guideline for treatment of TB and RR/MDR-TB in Suriname was updated following these latest recommendations of the WHO, and does not include ciprofloxacin and streptomycin anymore because of limited evidence of their effectiveness and sterilizing activity.

The clinical relevance of disputed mutations with low-level rifampicin resistance has been an issue of debate. One study indicated that disputed mutations had the same poor clinical prognosis as the most frequent undisputed mutations.[25] Williamson *et al.* suggested to conduct a multi-centre retrospective study to correlate the different types of *rpoB* mutations with clinical outcomes.[18] They stated that the retrospective nature of such a study would actually become a methodological strength, as it would allow data on treatment outcome to be obtained, and thus allow the clinical relevance of mutations to be assessed.[18] We consider our observational study to be such a study, that contains to our knowledge the largest set of patients infected with *M. tuberculosis* strains carrying the D435Y mutation. Our observation of good treatment results of patients with this peculiar type of low-level rifampicin resistance and susceptibility for isoniazid, suggests that treatment regimens with isoniazid and rifampicin can still be effective. The relatively high number of RR-TB cases with previous TB treatment is a concern and suggests that a sterilizing effect was not achieved. However, one-quarter of these patients did not complete the previous treatment which obviously contributed to relapse, while reinfection is also possible in an environment with ongoing transmission.

Several authors have proposed alternative treatment options for patients infected with a low-level rifampicin resistance, e.g. a higher dose of rifampicin or replacing rifampicin by rifabutin.[10,16,18,20,26] The currently recommended dose of rifampicin has been challenged over the last years.[27,28] Dosage studies indicate that 35 mg/kg rifampicin instead of 10 mg/kg is well tolerated and more effective than the standard dose.[29,30] These authors recommend high dose rifampicin for patients with TB meningitis, co-infection with HIV, diabetes mellitus and serious ill TB patients, since plasma concentrations of rifampicin are often too low. Our hypothesis is that patients with low-level rifampicin mono-resistant TB could benefit more from a first-line regimen, including isoniazid and high dose rifampicin (or rifabutin), rather than from a lengthy regimen for RR/MDR-TB treatment or the standard TB treatment. We have developed a study to treat these patients accordingly in Suriname and a protocol has been developed and cleared by the Human Scientific Research Ethic Committee, and includes informed consent of the patient for treatment with high-dose rifampicin (30 mg/kg); timely WGS testing of all RR-isolates; measuring of rifampicin blood levels; close supervision of treatment, including monitoring of side effects; case discussion in a Concilium; and a minimal follow-up of one year after treatment.

Our study also revealed several other issues, some beyond the scope of this study. All examined isolates of RR-TB patients, both found in Suriname and the Netherlands, belonged by WGS genetically to one large molecular transatlantic cluster. Transmission of the rifampicin-resistant strain is likely to be ongoing in Suriname, and possibly also in the Netherlands. Second, the observed TB/HIV co-infection rate (23%) is one of the highest in the WHO Region of the Americas [1]. More TB/HIV collaborative efforts are needed to control TB and move towards TB elimination in Suriname. RR-TB was more often detected in Creole people, which is also the ethnic group with a very high TB incidence, HIV co-infection rates and illicit drug use. Our data show a high TB case fatality of 14%, which has been associated with higher age and HIV co-infection.[31]

Our study had several strengths and limitations. We made use of a detailed electronic database and advanced molecular tests were applied on a subset of rifampicin-resistant *M. tuberculosis* isolates, although not all rifampicin-resistant isolates were available for additional typing. The 100% concordance of rifampicin resistance found by Xpert in Suriname and by WGS done in the Netherlands, confirmed that these patients actually had RR-TB. The major limitation of our study is that RR-TB patients were not actively followed up for recurrent TB after treatment completion. We will address this issue in the planned prospective study. Our analysis did not include rifampicin-susceptible isolates. Recently, we analysed another 118 lysates, including 18 rifampicin-resistant strains of patients diagnosed in 2018–2020. All rifampicin-resistant isolates had the D435Y mutation; none of the other strains carried mutations in the *katG* or *inhA* genes or had other first-line drug resistance-

associated mutations.

The findings of our study provide clarity to the rifampicin resistance situation in Suriname and possible implications for tailored treatment regimens. Our study shows that an epidemic of RR-TB was driven by a mutation of which the clinical significance is disputed. All but one strains examined revealed low-level rifampicin resistance and isoniazid susceptibility. Treatment results with rifampicin-containing regimens were unexpectedly good. Further research is needed to study alternative treatment options for these patients, such as regimens with high dose rifampicin for these specific group of patients, with systematic follow-up after treatment completion. We state that one size does not fit all for the treatment of RR/MDR-TB, i.e. some people need XL (extra-large) and others XS (extra-small) treatment regimens, depending on the causative strain. Technological advances make it increasingly possible to tailor treatment to the patient and their bacteria. Furthermore, collaborative networks of professionals and countries with different resources can work together to provide the necessary scientific evidence for effective XL or XS RR/MDR-TB treatment regimens. In the end, we want to provide each patient the right treatment, not too much and not too little, a concept of stratified medicine also advocated for the treatment of TB. [32]

Ethical statement

Ethical approval for this study was obtained from the Human Scientific Research Ethic Committee of the Ministry of Health of Suriname. The Dutch National TB Registration Committee approved the use of the data from the Netherlands for this study.

CRediT authorship contribution statement

F.A. Gopie: Conceptualization, Methodology, Writing - original draft. **E. Commiesie:** Conceptualization, Methodology, Writing - original draft. **S. Baldi:** Data curation, Writing - review & editing. **M. Kamst:** Data curation, Writing - review & editing. **D. Kaur:** Writing - review & editing. **W.C.M. de Lange:** Writing - review & editing. **P.S. Pinas:** Writing - review & editing. **D. Stijnberg:** Data curation, Writing - review & editing. **M. Wongsokarijo:** . **C.W.R. Zijlmans:** Writing - review & editing. **R. de Zwaan:** Writing - review & editing. **D. van Soolingen:** Writing - review & editing. **S.G.S. Vreden:** Supervision, Writing - review & editing. **G. de Vries:** Conceptualization, Formal analysis, Methodology, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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